

USAMRIID-NIAID-CDC

In Vitro Antiviral SARS-CoV Testing Program

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Roles of USAMRIID-NIAID-CDC in SARS Testing Program

◆ NIAID

- Negotiation of joint NIAID-USAMRIID Cooperative Research and Development Agreement (CRDA) signed by both NIAID and USAMRIID for SARS
- Collection and processing of compounds
- Implementation of program by NIAID contractor

◆ USAMRIID

- *In Vitro* Antiviral Testing
 - SARS associated Coronavirus
- Follow-up plaque-reduction, yield reduction
- Monkeypox, cowpox, vaccinia and camelpox testing available at USAMRIID and variola (smallpox) by USAMRIID at CDC



Therapeutic Targets

◆ Viral

- Cysteine protease
- RNA-dependent RNA polymerase
- Genome replication and transcription
- Fusion

◆ Other

- Immune response
- Lung pathology

◆ SARS-CoV growth only in Vero 76 & Vero E6 limits ability to test come potential therapies



Testing Priorities

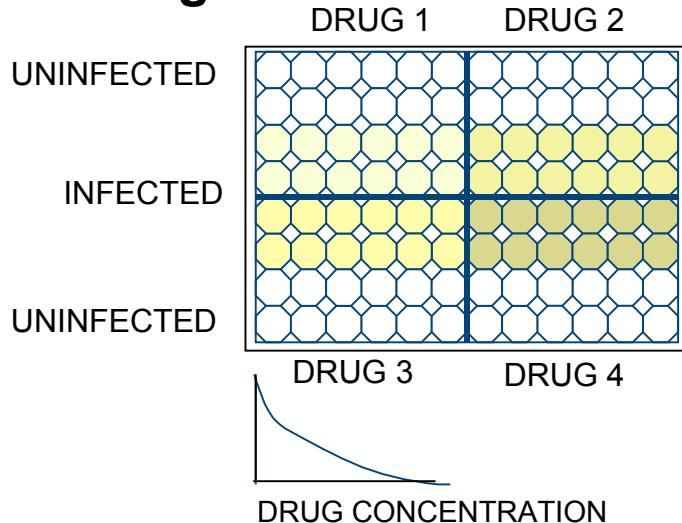
- ◆ FDA-approved anti-viral drugs
- ◆ Drugs in clinical development in one of the target categories
- ◆ Other FDA-approved drugs-Preswick library (eg. antibiotics)
- ◆ Other compounds



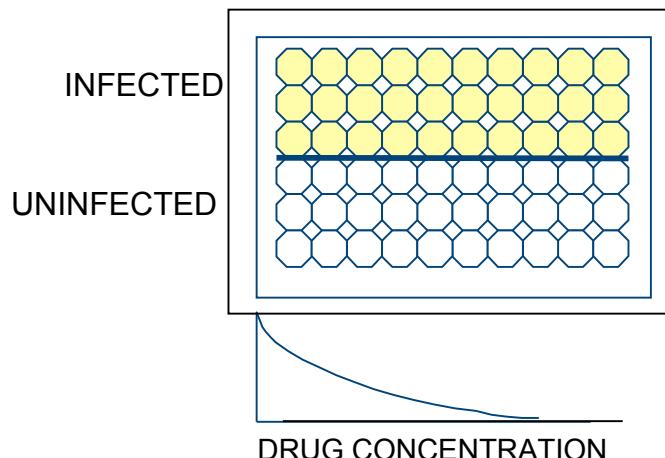
SARS Antiviral Assay

CPE Based Neutral Red Uptake

Initial Testing



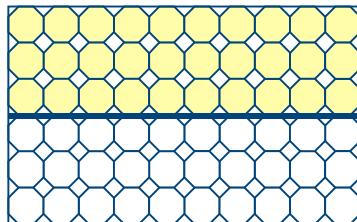
Confirmatory Testing



- 4 Drugs screened/plate
- 5-Fold dilution
- 5 Dilutions
- 625 fold range
- Typically 100 to 0.1 ug/ml
- 1 Cell line Vero 76
- 1 Drug screened/plate
- 3-Fold dilution
- 8 Dilutions
- 2187 fold range
- Typically 100 to 0.05 ug/ml
- 2 Cell lines Vero 76 & E6



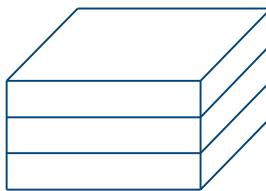
SARS Drug Screen Assay



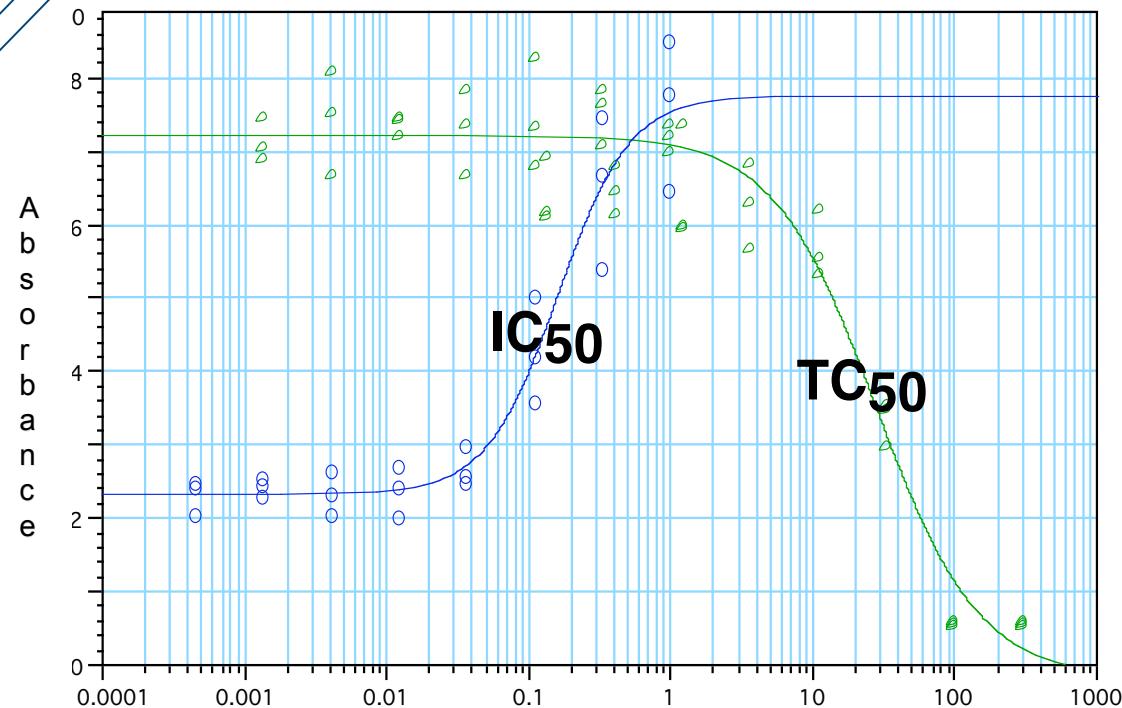
Infect with SARS
associated
coronavirus
200300592

Incubate
3 Days

- Assay Viability by Neutral Red Uptake
 - Pulse with neutral red for 90 minutes
 - Wash monolayer twice
 - Fix monolayer with 10% buffered formalin
 - Solubilize 50% Ethanol, pH 3.5
 - Read Plate



MOI 0.001 pfu/cell



Drug Concentration

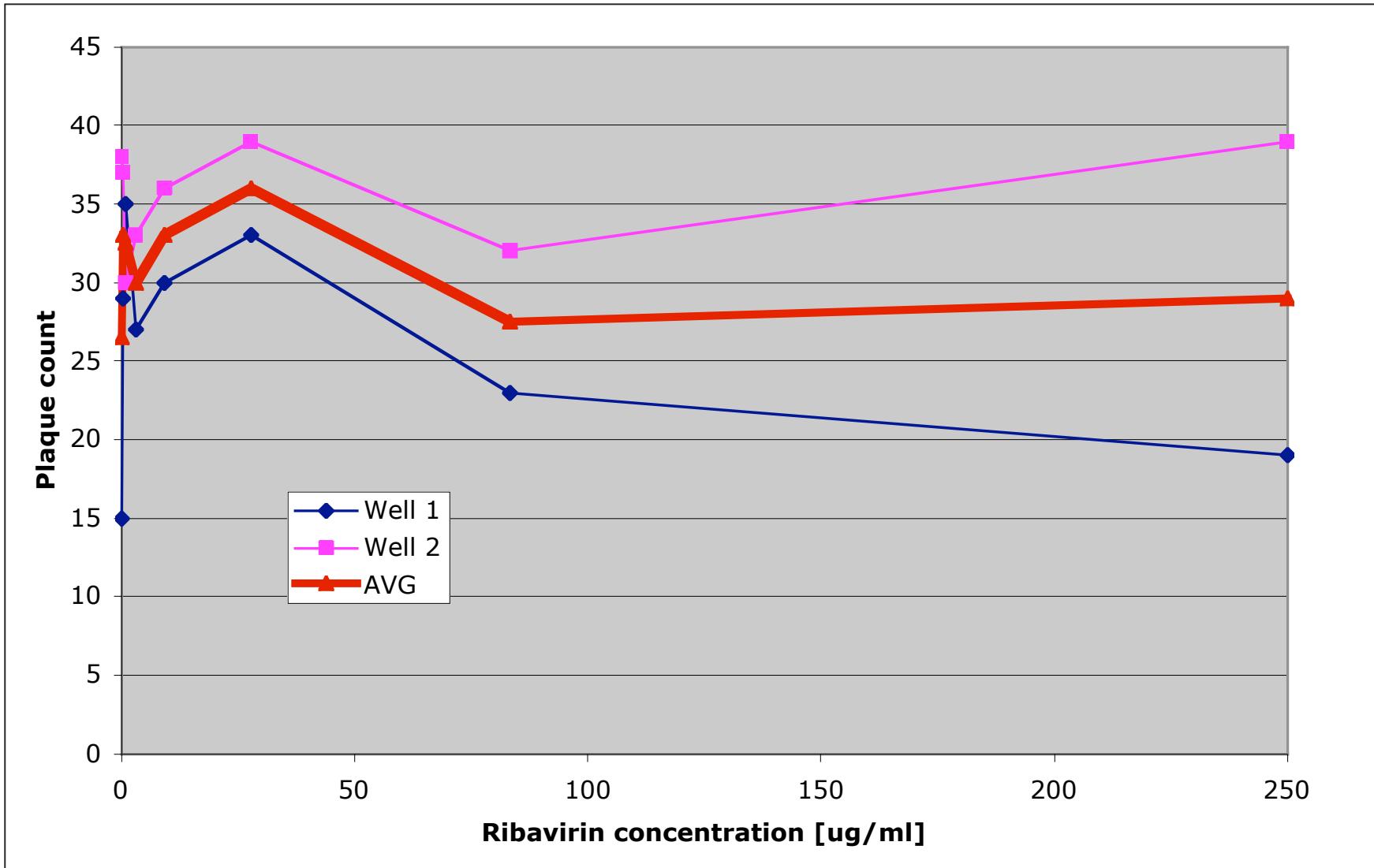


Ribavirin inhibition of SARS associated Coronavirus 200300592

MOI	VERO E6	VERO 76
0.01	>250 ug/ml	>250 ug/ml
0.005	>250 ug/ml	>250 ug/ml
0.0025	>250 ug/ml	>250 ug/ml
0.00125	>250 ug/ml	>250 ug/ml
0.0006	>250 ug/ml	>250 ug/ml



Ribavirin Plaque Reduction



Sensitivity to Ribavirin

<u>Virus</u>		EC_{50} (μ g/ml)	Cell Line	Protection in Animal Model
Bunyaviridae				
Phlebovirus	Rift Valley Fever	40	VERO 76	Mouse, Monkey
Phlebovirus	Sandfly Fever	5	VERO 76	No model
Nairovirus	Crimean-Congo Hemorrhagic Fever	8	VERO 76	Mouse
Hantavirus	Hemorrhagic Fever with Renal	15	VERO E6	Mouse
Togaviridae				
Flavivirus	Yellow Fever	250-500	VERO 76	Not active Monkey
Flavivirus	Dengue 1-4	200-400	VERO 76	Not active Monkey
Arenaviridae				
Arenavirus	Lassa	20	VERO 76	Guinea pig, Monkey
Arenavirus	Junin	22	VERO 76	Guinea pig, Monkey
Arenavirus	Machupo	32	VERO 76	Guinea pig, Monkey
Arenavirus	Guanarito	14	VERO 76	Guinea pig
Filoviridae				
Filovirus	Ebola	>500	VERO 76	Not active Mouse, Monkey
SARS Associated Coronavirus		>250 >250	Vero 76 Vero E6	Primate model development underway at USAMRIID



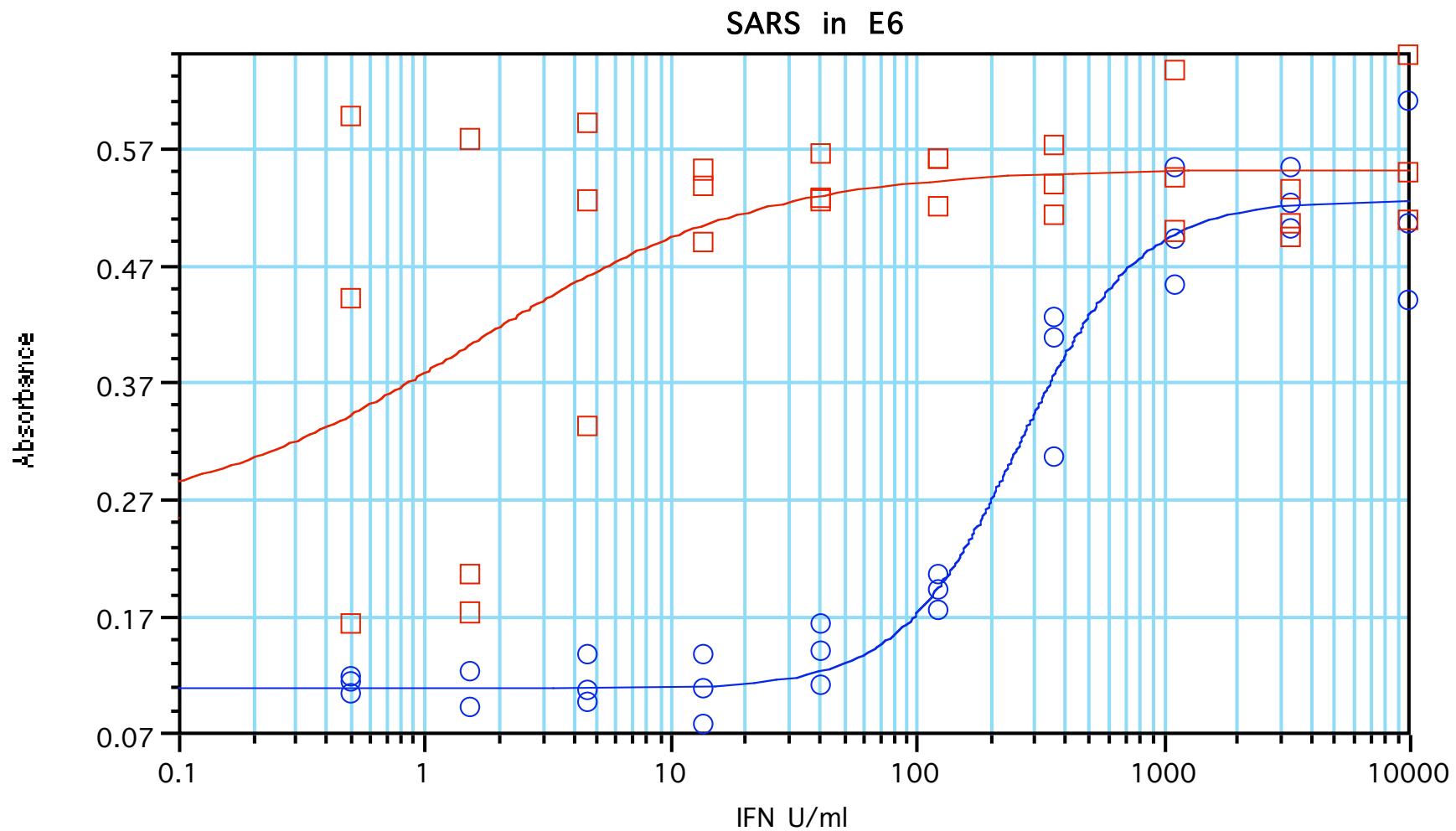
Activity of Some Interferons

- Vero E6 cell pre-incubated with interferon overnight
- MOI 0.01

Interferon	Activity IU/ml
IFN Alpha A	22900
IFN Alpha D	2339
IFN Alpha A/D	2976
IFN Beta	261, 405
IFN Gamma	>10,000



INF Beta



$$y = \frac{(A - D)}{(1 + (x/C)^B)} + D; \quad A = 0.108, \quad B = 1.784, \quad C = 261.322, \quad D = 0.525, \quad R^2 = 0.967$$

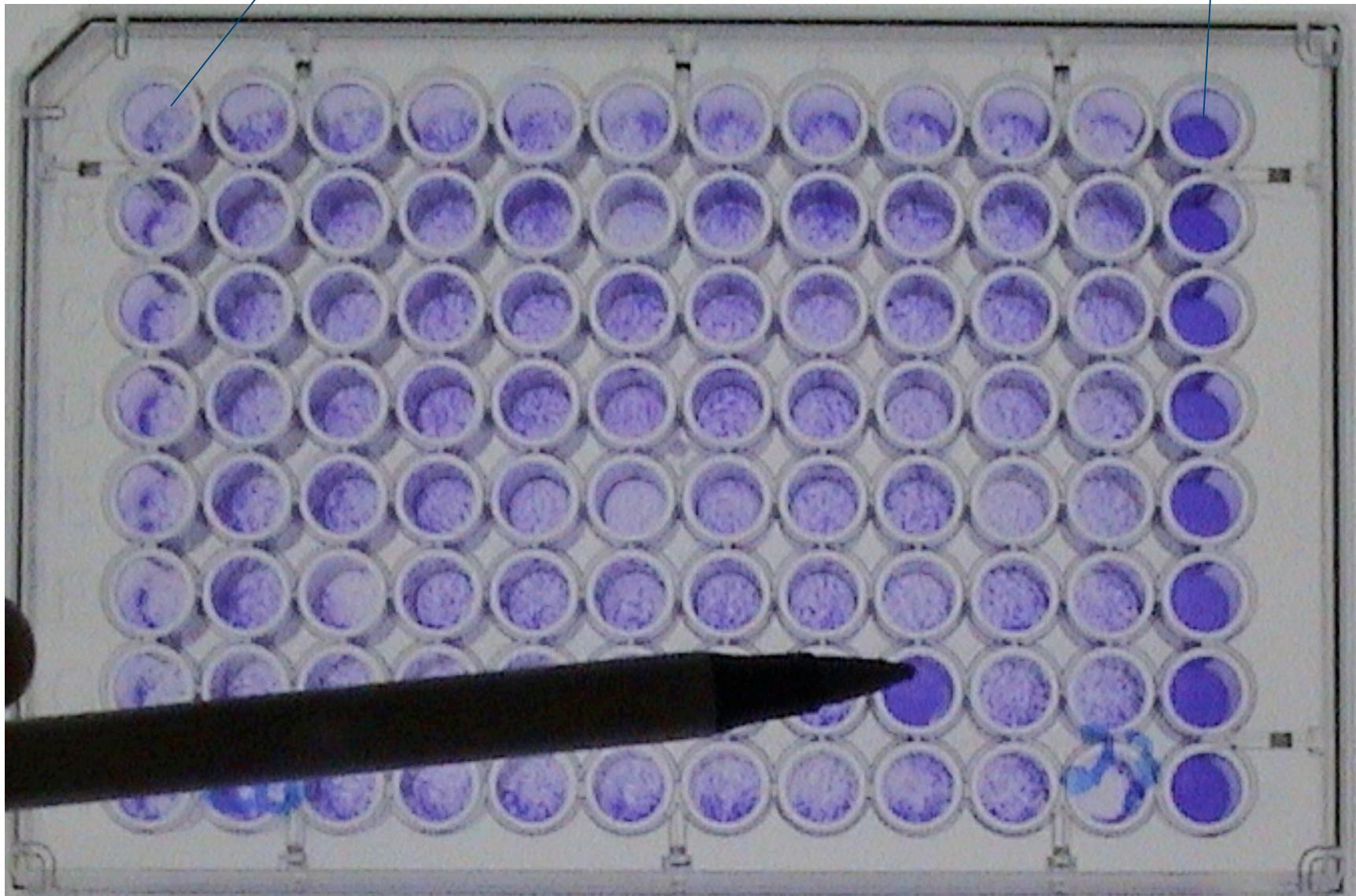
- SARS E6 IFN-Beta (Infected: Concentration vs Values)
- Toxicity (uninfected: Concentration vs Values)



Rapid Screen for Large Collections

Virus Only

No virus



Results

Anti-SARS activity

- Alpha & Beta Interferon
- Rimantadine
- Cysteine Protease Inhibitors

None yet with clinical applicability

Not all active compound can be made public at this time. Depends on wishes of compound owner.



Animal Model Development

- o Intranasal infection of primates produces lung pathology consistent with SARS
- o Intravenous infection(3×10^6 pfu) produced lethal disease in cynomolgus monkeys (1 of 1)
- o Small rodent model could be useful
 - o Cotton rats ?
 - o Mouse hepatitis virus as surrogate?
- o Animal model may be required if number of drug candidates exceed capacity to evaluate in clinical trials



Human Coronavirus Strain OC 43 Assay in BS-C-1 Cells

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Supported by: NIAID, Virology Branch



Human Coronavirus Assay

- Cells : African green monkey kidney cells (BS-C-1)
- Virus: human coronavirus (strain OC 43)
- Assays
 - Cytopathic Effect Reduction Assay
 - Neutral Red Uptake Assay
 - Virus Yield Reduction Assay
- MOI
 - 0.01 (3 day test)
 - 0.001 (6 day test)

Antiviral SARS-CoV Testing Program Staff

◆ USAMRIID (Virologists)

- John Huggins, Ph.D.
- Robert Baker, Ph.D.
- David Miller, B.S.
- Debbie Kefauver
- Susan Zwiers, M.S.
- Erick Muller, M.S.
- Chris Hartmann, M.S.

◆ SRI (Medicinal Chemists)

- John A. Secrist III, Ph. D.
- Sam Ananthan, Ph. D.
- Cecil D. Kwong, Ph. D.
- Joseph A. Maddry, Ph. D.
- Robert C. Reynolds, Ph. D.

◆ NIAID (Drug Development Support)

- Catherine Laughlin, Ph.D.
- Christopher Tseng, Ph.D.
- Walla Dempsey, Ph.D.
- Teri Greenfield, M.S.

- Primary Contact is NIAID Repository Contractor

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- Questions about antiviral testing

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